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**A STEPWISE ONE POT SYNTHESIS OF
ALKYL THIOPHOSPHORAMIDATE
DERIVATIVES OF NUCLEOSIDES**

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ABSTRACT

Novel alkyl thiophosphoramidate derivatives of nucleoside analogues as membrane-soluble prodrugs of the bioactive free nucleotides have been prepared by phosphochloridothioate chemistry.

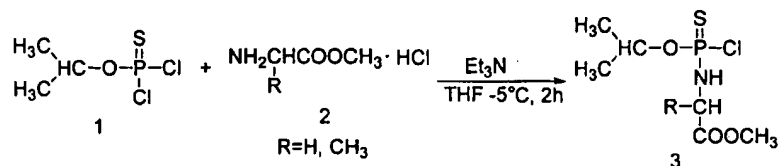
Phosphate triester derivatives of nucleosides have been prepared as membrane-soluble pro-drugs of the bio-active nucleotides, and have been evaluated against HIV-1 in vitro. Nucleoside analogues act only after intracellular conversion to their 5'-triphosphate.¹ This dependence of nucleoside analogues on the host kinase can be a major limitation which cannot easily be overcome by the use of simple nucleotides, as their charge greatly impedes membrane penetration.² Consequently, there was much interest

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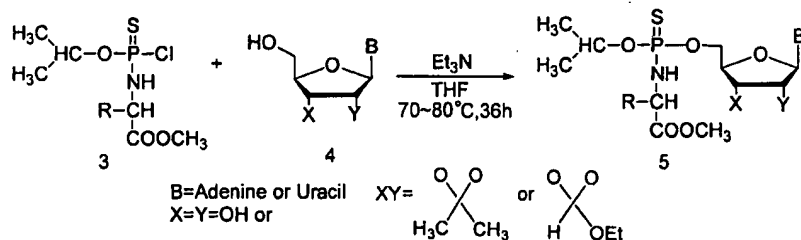
in the use of masked phosphate esters as membrane-soluble depot forms of the bio-active nucleotides for chemotherapeutic nucleoside analogues.³⁻⁶ Recently McGuigan reported that phosphate triester derivatives of AZT bearing amino acid moieties did have anti-HIV activity.⁷

The relative metabolic stability of nucleoside-5'-phosphorothioates is well-documented. For instance, AMP-S is relatively resistant to enzymatic transformations by adenylate deaminase, adenylate kinase, and 5'-nucleotidase. And ATP- α -S diastereoisomers exhibit selective metabolic stability.⁸⁻⁹

For the purpose of developing new types of prodrugs, in this paper we report an efficient method of synthesizing different alkyl thiophosphoramidate derivatives of nucleosides. The target compounds were synthesized as shown in Schemes 1 and 2. *O*-Isopropyl phosphorodichloridothioate (1) was used as a starting material. The key step was the coupling of nucleosides or their analogues with alkyl methoxyaminoacyl thiophosphorochloridate (3) to form new conjugated compounds (5).



Scheme 1.



Scheme 2.

A reaction of amino acid methyl ester (2) with *O*-isopropyl phosphorodichloridothioate (1) was performed at -5°C under nitrogen atmosphere (Scheme 1). Triethylamine was added via syringe to the stirring solution. The reaction was monitored by ^{31}P NMR spectroscopy. It was

found that *O*-isopropyl phosphorodichloridothioate (1) with a ^{31}P NMR shift at 56.47 ppm was transferred into 2 at about 70 ppm within approximately 2 h, then a solution of nucleoside or its analogue (4) and triethylamine in pyridine or THF was added to the reaction solution (Scheme 2). After 34 h at 70 ~ 80°C the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in chloroform and washed with 1 M hydrochloric acid solution, saturated sodium bicarbonate solution and then water. The organic phase was dried (MgSO_4) and evaporated under vacuum, and the residue was purified by chromatography on silica by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave the product (5) in 81–91% yields.

Although unprotected nucleosides, for example uridine and adenosine, were used, phosphorylation took place selectively at the 5'-position (^1H and ^{13}C NMR). Alkyl thiophosphoramidate derivatives of nucleoside (5) were obtained as a mixture of diastereoisomers due to the chirality at the phosphorus center. Hence the ^{31}P NMR chemical shifts appeared as a pair of peaks at about 60 ppm. The dissolution of the nucleoside is essential for the occurrence of the reaction. Table 1 lists the products of alkyl thiophosphoramidate derivatives of nucleosides (5).

Formation of 5a was traced by ^{31}P NMR spectroscopy shown in Figures 1 and 2. The starting material *O*-isopropyl phosphorodichloridothioate (1) in THF shows ^{31}P NMR at 56.47 ppm, after the solution of amino acid methyl ester hydrochloride (2) and triethylamine was added to the solution of (1), the peak at ^{31}P NMR 56.47 ppm disappeared in 2 h with a pair of new peaks at ^{31}P NMR 71.01 ppm and 69.83 ppm emerging corresponding to compound 3a (Figure 1). When the nucleoside or its analogue (4) was added, the double peaks at 60.13 and 59.26 ppm appeared corresponding to compound 5a (Figure 2). After 34 h, only a pair of peaks at

Table 1. Products of Alkyl Thiophosphoramidate Derivatives of Nucleosides

5	R	Nucleoside (4)	Yield (%)
5a	H	Adenosine	83.9
5b	CH_3	Adenosine	87.4
5c	H	Uridine	81.2
5d	CH_3	Uridine	85.1
5e	H	2',3'- <i>O</i> -Isopropylidene uridine	90.6
5f	CH_3	2',3'- <i>O</i> -Isopropylidene uridine	81.7
5g	CH_3	2',3'- <i>O</i> -Ethoxymethylidene adenosine	85.6

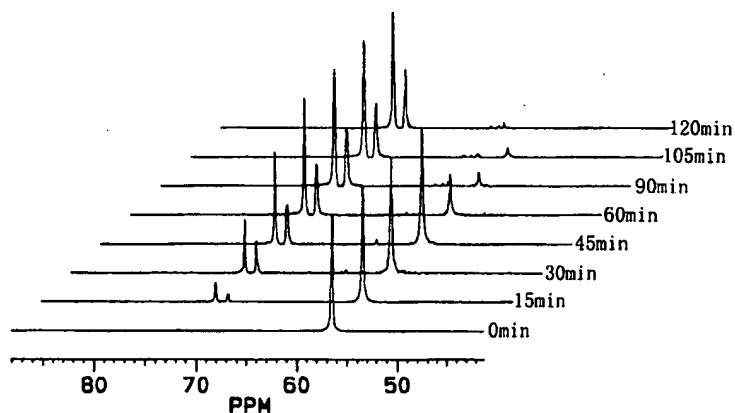


Figure 1. The stack ^{31}P NMR spectra of formation of compound 3a.

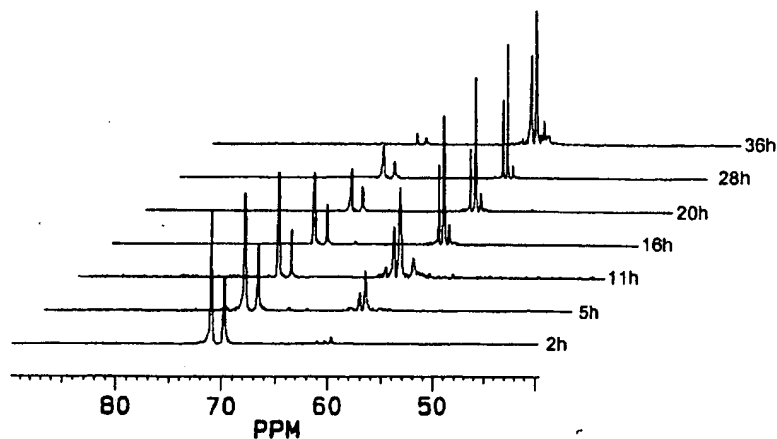


Figure 2. The stack ^{31}P NMR spectra of formation of compound 5a.

about ^{31}P NMR 60 ppm was observed. Triethylamine acted as a catalytic reagent, besides capturing hydrochloride produced in the reactions.

In conclusion, in this paper a convenient and efficient approach to synthesis of alkyl thiophosphoramidates derivatives of nucleosides under mild conditions has been developed. In the first step only one chloride of *O*-isopropyl-phosphorodichloridothioate is displaced by the amino acid ester to form a new phosphorus–nitrogen bond. Nucleoside thiophosphor-

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ylation with high selectivity takes place at the 5'-position rather than at 2'- or 3'-position. The reaction is a convenient two-step one pot synthesis and the intermediate need not be separated in the middle of reaction. More detailed investigations of these compounds and their biological activity are currently underway.

EXPERIMENTAL

General Information

All glassware was dried in an oven for at least 3 h at 120°C prior to use. Air sensitive materials were transferred under a nitrogen atmosphere. THF and triethylamine were dried over Na and CaH₂ respectively. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AM 500 spectrometer. TMS (δ = 0.0) and CDCl₃ (δ = 7.24 ppm) were references for ¹H and ¹³C NMR spectra respectively. ¹³C NMR spectra were all taken under ¹H decoupled and ³¹P coupled conditions. ³¹P NMR spectra were taken on a Bruker AC 200 spectrometer at 81 MHz under ¹H decoupled conditions. ³¹P NMR chemical shifts were reported in ppm downfield (+) or upfield (−) from external 85% H₃PO₄ as reference. Mass spectra were conducted on a Bruker Esquire-LC mass spectrometer operated in positive and negative ion mode.

Synthesis of Amino Acid Methyl Ester Hydrochloride (2) and Protected Nucleoside (4)

The preparations of amino acid methyl ester hydrochloride (2) and protected nucleoside (4) were carried out according to the literature.¹⁰⁻¹² All physical constants and spectroscopies data of the products synthesized were in agreement with the literature.

General Procedure for Synthesis of Alkyl Thiophosphoramidate Derivatives of Nucleosides

A solution of triethylamine (1.4 ml, 1.0 g, 10.0 mmol) in THF (10 ml) was added dropwise with vigorous stirring to a solution of amino acid methyl ester hydrochloride (2, 5.02 mmol) and *O*-isopropyl phosphorodichloridothioate (1, 0.97 g, 5.02 mmol) in THF (10 ml) at −5°C over a period of 15 min. The reaction mixture was slowly warmed to ambient

temperature with stirring over 2 h, and the solvent was then removed in vacuum. The residue was treated with THF (15 ml), the mixture was filtered, and the filtrate was evaporated in a vacuum to yield the product (3) as a colorless oil (5.02 mmol, 100%). A solution of nucleoside (4, 5.02 mmol) was dissolved in pyridine (10 ml), and alkyl methoxyalaninyl thiophosphorochloridate (3) (5.02 mmol) and triethylamine (0.7 ml, 0.5 g, 5.02 mmol) were added with vigorous stirring. After 34 h at 70~80°C the solvent was removed under vacuum. The residue was dissolved in chloroform (10 ml) and washed with 1 M hydrochloric acid solution (2 × 15 ml), saturated sodium bicarbonate solution (2 × 10 ml), and then water (3 × 15 ml). The organic phase was dried (MgSO₄) and evaporated under vacuum, and the residue was purified by chromatography on silica by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave the product 5.

Compound 5a (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent (*R_f*=0.73 for TLC). 2.00 g (yield 83.9%). ³¹P NMR (CDCl₃, δ: ppm, *J*: Hz): δ 60.13, 59.26; ¹H NMR (500 MHz, CDCl₃): δ 8.22 (1H, s, H-8), 8.07 (1H, s, H-2), 7.36 (2H, bs, NH₂), 6.61, 6.60 (1H, d, ³*J*=6.0, H-1'), 5.59 (1H, br, 2'-OH), 5.44 (1H, br, 3'-OH), 5.16 (1H, s, P-NH), 4.69 (1H, m, H-2'), 4.54 (1H, m, OCHMe₂), 4.41 (1H, m, H-3'), 4.16 (1H, m, H-4'), 3.80 (2H, m, H-5'), 3.66 (2H, m, H-α), 3.51 (3H, s, OCH₃), 1.02 (6H, m, OCH(CH₃)₂); ¹³C NMR (500 MHz, CDCl₃): δ 173.54 (COOMe), 152.53 (C-2), 150.28 (C-6), 143.60 (C-4), 138.22 (C-8), 121.07 (C-5), 91.28 (C-4'), 87.68 (C-1'), 82.35 (C-3'), 80.24 (C-2'), 64.15 (C-5'), 61.51 (C-α), 57.07 (OCH₃), 44.37 (OCH(CH₃)₂), 21.60, 21.54 (OCH(CH₃)₂); ESI-MS (pos.): *m/z* 477 (M + H)⁺; ESI-MS (neg.): *m/z* 475 (M - H)⁻.

Compound 5b (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent (*R_f*=0.77 for TLC). 2.15 g (yield 87.4%). ³¹P NMR (CDCl₃, δ: ppm, *J*: Hz): δ 59.11, 58.23; ¹H NMR (500 MHz, CDCl₃): δ 8.16 (1H, s, H-8), 8.01 (1H, s, H-2), 7.40 (2H, bs, NH₂), 6.44, 6.43 (1H, d, ³*J*=6.0, H-1'), 5.57 (1H, br, 2'-OH), 5.41 (1H, br, 3'-OH), 5.24 (1H, s, P-NH), 4.64 (1H, m, H-2'), 4.56 (1H, m, OCHMe₂), 4.31 (1H, m, H-3'), 4.18 (1H, m, H-4'), 3.83 (2H, m, H-5'), 3.78 (1H, m, H-α), 3.60 (3H, s, OCH₃), 1.08, 1.07 (3H, d, ³*J*=6.0, β-CH₃), 0.95 (6H, m, OCH(CH₃)₂); ¹³C NMR (500 MHz, CDCl₃): δ 174.24 (COOMe), 158.63 (C-2), 155.08 (C-6), 151.30 (C-4), 143.28, 143.16 (C-8), 122.07 (C-5), 90.98 (C-4'), 88.38 (C-1'), 76.35 (C-3'), 73.24 (C-2'), 64.15 (C-5'), 63.70 (C-α), 52.07 (OCH₃), 43.41 (OCH(CH₃)₂), 26.35 (C-β), 19.50, 19.44 (OCH(CH₃)₂); ESI-MS (pos.): *m/z* 491 (M + H)⁺; ESI-MS (neg.): *m/z* 489 (M - H)⁻.

Compound 5c (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent (*R_f*=0.68 for TLC). 1.85 g (yield 81.2%). ³¹P NMR (CDCl₃, δ: ppm, *J*: Hz): δ 61.65, 60.89; ¹H NMR (500 MHz, CDCl₃): δ 11.38 (1H, br, H-3),

7.86, 7.85 (1H, d, $^3J=5$, H-6), 5.87 (2H, m, H-1', 5), 5.51 (1H, br, 3'-OH), 5.33 (1H, br, 2'-OH), 4.66 (1H, m, OCHMe₂), 4.33 (2H, m, H-2', 3'), 4.21 (1H, m, H-4'), 4.07 (2H, m, H-5'), 3.80 (2H, m, H- α), 3.74 (3H, s, OCH₃), 3.32 (1H, m, P-NH), 1.10 (6H, m, OCH(CH₃)₂); ^{13}C NMR (500 MHz, CDCl₃): δ 173.24 (COOMe), 163.70 (C-2), 150.43 (C-4), 136.11 (C-6), 109.36 (C-5), 87.23 (C-1'), 83.76 (C-4'), 82.36 (C-2'), 70.42 (C-3'), 66.72 (OCH(CH₃)₂), 61.33 (C-5'), 54.79 (OCH₃), 46.82 (C- α), 23.75 (OCH(CH₃)₂); ESI-MS (pos.): m/z 454 (M + H)⁺; ESI-MS (neg.): m/z 452 (M - H)⁻.

Compound 5d (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent ($R_f=0.71$ for TLC). 1.99 g (yield 85.1%). ^{31}P NMR (CDCl₃, δ : ppm, J : Hz): δ 61.24, 60.38; ^1H NMR (500 MHz, CDCl₃): δ 11.43 (1H, br, H-3), 8.00, 7.99 (1H, d, $^3J=5$, H-6), 5.88 (2H, m, H-1', 5), 5.51 (1H, br, 3'-OH), 5.37 (1H, br, 2'-OH), 4.86 (1H, m, OCHMe₂), 4.37 (2H, m, H-2', 3'), 4.28 (1H, m, H-4'), 4.17 (2H, m, H-5'), 3.85 (2H, m, H- α), 3.64 (3H, s, OCH₃), 3.33 (1H, m, P-NH), 1.29 (6H, m, OCH(CH₃)₂), 1.08, 1.07 (3H, d, $^3J=6.0$, β -CH₃); ^{13}C NMR (500 MHz, CDCl₃): δ 178.33 (COOMe), 165.26 (C-2), 148.33 (C-4), 134.61 (C-6), 111.46 (C-5), 87.25 (C-1'), 84.26 (C-4'), 83.86 (C-2'), 71.42 (C-3'), 70.44 (OCH(CH₃)₂), 64.83 (C-5'), 52.99 (OCH₃), 46.82 (C- α), 26.45 (C- β), 23.75 (OCH(CH₃)₂); ESI-MS (pos.): m/z 468 (M + H)⁺; ESI-MS (neg.): m/z 466 (M - H)⁻.

Compound 5e (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent ($R_f=0.88$ for TLC). 2.24 g (yield 90.6%). ^{31}P NMR (CDCl₃, δ : ppm, J : Hz): δ 62.43, 61.56; ^1H NMR (500 MHz, CDCl₃): δ 9.11, 9.10 (1H, d, $^3J=5.5$, H-3), 7.59, 7.58 (1H, d, $^3J=4.5$, H-6), 5.82 (2H, m, H-1', 5), 4.93 (2H, m, H-2', 3'), 4.66 (1H, m, OCHMe₂), 4.42 (1H, m, H-4'), 4.00 (2H, m, H-5'), 3.63 (3H, s, OCH₃), 3.57 (2H, m, H- α), 3.35 (1H, m, P-NH), 1.53 (3H, s, CH₃), 1.33 (3H, s, CH₃), 1.30 (6H, m, OCH(CH₃)₂); ^{13}C NMR (500 MHz, CDCl₃): δ 171.25 (COOMe), 169.02 (C-4), 141.99 (C-2), 145.39, 145.36 (C-6), 117.02, 116.96 (>CMe₂), 104.33, 104.22 (C-5), 95.83, 95.16 (C-1'), 87.97 (C-4'), 87.20, 87.13 (C-2'), 83.64, 83.51 (C-3'), 72.44, 72.41 (OCH(CH₃)₂), 67.24 (C-5'), 55.09 (OCH₃), 45.80, 45.73 (C- α), 28.63 (CH₃), 26.86 (CH₃), 23.36 (CH₃); ESI-MS (pos.): m/z 494 (M + H)⁺; ESI-MS (neg.): m/z 492 (M - H)⁻.

Compound 5f (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent ($R_f=0.86$ for TLC). 2.08 g (yield 81.7%). ^{31}P NMR (CDCl₃, δ : ppm, J : Hz): δ 61.90, 61.16; ^1H NMR (500 MHz, CDCl₃): δ 9.56 (1H, br, H-3), 7.88, 7.87 (1H, dd, $^3J=5$, H-6), 5.89 (2H, m, H-1', 5), 4.98 (2H, m, H-2', 3'), 4.63 (1H, m, OCHMe₂), 4.41 (1H, m, H-4'), 4.04 (2H, m, H-5'), 3.89 (1H, m, P-NH), 3.73 (4H, m, OCH₃, H- α), 1.61 (3H, s, CH₃), 1.42 (3H, s, CH₃), 1.31, 1.30 (3H, d, $^3J=6$, β -CH₃), 1.12 (6H, m, OCH(CH₃)₂); ^{13}C NMR (500 MHz, CDCl₃): δ 174.27 (COOMe), 163.13 (C-4), 151.28 (C-2), 144.36, 144.32 (C-6), 118.40, 117.59 (>CMe₂), 107.62, 107.43 (C-5), 98.76,

98.41 (C-1'), 86.23 (C-4'), 85.71, 85.63 (C-2'), 82.70, 82.58 (C-3'), 71.93 (OCH(CH₃)₂), 65.44 (C-5'), 56.87 (OCH₃), 50.73, (C-α), 48.66 (C-β), 26.53 (CH₃), 25.85 (CH₃), 23.68 (CH₃); ESI-MS (pos.): *m/z* 508 (M + H)⁺; ESI-MS (neg.): *m/z* 506 (M - H)⁻.

Compound 5g (diastereoisomers): MeOH:CHCl₃ (1:20) as eluent (*R_f*=0.85 for TLC). 2.35 g (yield 85.6%). ³¹P NMR (CDCl₃, δ: ppm, *J*: Hz): δ 58.46, 57.90; ¹H NMR (500 MHz, CDCl₃): δ 8.67 (1H, s, H-8), 8.58 (1H, s, H-2), 7.41 (2H, br, NH₂), 6.44, 6.43 (1H, d, ³*J*=5.0, H-1'), 6.14 (1H, s, >CHOEt), 5.52 (1H, m, H-2'), 5.14 (1H, m, H-3'), 4.56 (1H, m, OCHMe₂), 4.41 (1H, m, H-4'), 3.74 (3H, m, H-5', H-α), 3.50 (5H, m, OCH₃, OCH₂CH₃), 1.16, 1.15 (3H, d, ³*J*=6.0, β-CH₃), 1.12 (6H, m, OCH(CH₃)₂), 0.97 (3H, m, OCH₂CH₃); ¹³C NMR (500 MHz, CDCl₃): δ 171.54 (COOMe), 152.13 (C-2), 149.14 (C-6), 143.81 (C-4), 133.45 (C-8), 128.18 (C-5), 117.85 (>CHOEt), 91.24 (C-4'), 86.70 (C-1'), 84.31 (C-3'), 81.78 (C-2'), 72.12 (OCH(CH₃)₂), 65.07 (C-5'), 64.77 (OCH₃), 52.58 (>CHOCH₂CH₃), 50.43 (C-α), 23.55, 23.36 (OCH(CH₃)₂), 19.53 (C-β), 14.18 (>CHOCH₂CH₃); ESI-MS (pos.): *m/z* 547 (M + H)⁺; ESI-MS (neg.): *m/z* 545 (M - H)⁻.

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